Review Article

CAR-T Cell Therapy: Managing Side Effects and Overcoming Challenges

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Abstract

Chimeric antigen receptor T (CAR-T) cell therapy is an innovative and promising approach to treat cancer. Clinical trials have demonstrated remarkable results, providing hope for patients who have exhausted more traditional therapies. However, this new therapy is not without challenges, as significant side effects have been associated with it. Cytokine release syndrome (CRS) is a widely recognized and consequential side effect of CAR-T cell therapy. Neurological toxicity is another potential side effect that can cause confusion and seizures in some patients. Hematologic toxicities, such as anemia and thrombocytopenia, can increase the risk of bleeding or infection. B-cell aplasia can also occur, leading to increased vulnerability to infections. Strategies to reduce the incidence and severity of toxicities include suicide, endogenous, and exogenous switches to modulate the activity of the immune system toward cancer while minimizing toxicity. Despite the obstacles faced by CAR-T cell therapy, continuous research and development in this area offer considerable potential for improving this treatment as a more reliable and efficient method for treating cancer.

Keywords: CAR-T cell, cell therapy, challenge, immunotherapy, side effects

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Submitted: 25-Dec-2023; **Revised:** 06-Sep-2024; **Accepted:** 25-Sep-2024; **Published:** 30-Apr-2025

Introduction

Targeted immunotherapy is considered one of the optimal cancer therapies among various treatment methods.[1] This treatment is achieved when tumor cells are eradicated without damaging healthy tissue or causing the toxicity associated with conventional chemotherapy.[2,3] The history of producing these cells dates back to less than two decades ago, when numerous influential monoclonal antibodies were introduced to treat cancer.[4] Despite the remarkable success of immunotherapy using monoclonal antibodies against specific cancer antigens, there is a short period of stability for these antibodies in the patient's bloodstream.[5] These cells have proven successful and promising in clinical studies, particularly when used in lymphoblastic leukemia and Hodgkin's lymphoma.[6,7] They have armed T lymphocytes with a chimeric antigen receptor (CAR), similar to the active part of the antibody, by modeling the amino acid structure of the monoclonal antibody.[8] These cells possess both the abilities of T lymphocytes and the specificity of monoclonal antibodies to kill target cells.[9,10] Recently, studies have expanded CAR T cell therapy beyond cancer to include cardiometabolic disorders, autoimmune diseases, fibrosis, and cellular senescence. Infectious diseases, once early targets, are also being revisited with improved understanding of immune responses and enhanced techniques, such as advanced CAR constructs, better manufacturing, and targeting various immune cell subsets like Tregs and macrophages.

While CAR-T cell therapy was first proposed in the late 1980s, it took several decades of research and development before

Access this article online

Quick Response Code:

Website: https://journals.lww.com/ADBM

DOI: 10.4103/abr.abr_531_23

How to cite this article: Golmohammadi M, Noorbakhsh N, Kavianpour M. CAR-T cell therapy: Managing side effects and overcoming challenges. Adv Biomed Res 2025;14:38.

the first generation of CAR-T cells was successfully used in clinical trials in the early 2000s.[11,12] To put it differently, the CAR-T cells of the first generation were created by genetically modifying T cells. The purpose of this modification was to enable these cells to display a specialized receptor known as CAR on their surface. The CARs were designed to selectively recognize a unique protein present only in cancer cells.[13] The second and third generations of CAR-T cells were designed with additional co-stimulatory molecules to enhance their activity and prolong their survival in the body.[14] The fourth generation of CAR-T cells, also known as TRUCKs (T-cells redirected for universal cytokine killing), is designed to secrete cytokines or other molecules that can stimulate the immune system and enhance the anti-tumor response. To accomplish this, the cells are modified to contain a section that is responsive to the nuclear factor of activated T-cells (NFAT), which includes a transgenic cytokine like IL-12.[15] When the CD3ζ-containing CARs interact with their designated target, the transgene is activated. To engineer TRUCK CAR-T cells, two transgenic cassettes are typically transferred: one for the CAR structure and another for the inducible cytokine.[16] Currently, the fifth generation of CAR-T cells is still in the early stages of development. Proposed modifications include the incorporation of gene editing techniques like CRISPR to improve the precision and specificity of CAR-T cells, as well as the use of synthetic biology techniques to create more complex cellular circuits that can respond to multiple signals in the tumor microenvironment.[17] Ongoing investigations and advancements in this area of research could lead to additional enhancements in the efficacy of CAR-T cell treatment for cancer. This type of therapy offers several advantages that make it an appealing treatment, including the ability to identify target cells independent of the human leukocyte antigens (HLA) system, kill cancer cells that utilize tumor escape mechanisms, produce large populations of tumor-specific T cells in patients, and minimally damage healthy tissues and organs.[18,19] More importantly, shorter treatment times and fewer side effects are significant achievements of CAR-T cell therapy. While these cells are promising, significant challenges remain concerning their safety and efficacy, despite the encouraging results seen in clinical trials. Specifically, while CAR-T cells have demonstrated impressive anti-tumor activity, most clinical responses have been associated with toxicity, and some patients have even experienced fatal complications following treatment.[20] In light of these concerns, a review article was recently published that aimed to survey CAR-T cell-related toxicities and explore both traditional and new methods to overcome these barriers. We emphasized the importance of understanding the mechanisms underlying CAR-T cell toxicity, as this knowledge is essential for developing effective interventions to mitigate adverse effects.

Side Effects of CAR-T Cell Therapy

The therapeutic benefits of CAR-T cell therapy are often accompanied by toxic side effects, some of which can be life-threatening.[21] These toxicities can affect the cardiovascular, respiratory, and neurological systems and may manifest in various ways, such as cytokine release syndrome (CRS), tumor lysis syndrome (TLS), B-cell aplasia, graft-versus-host disease (GVHD), uncontrolled proliferation of CAR-T cells, genotoxicity, and neurotoxicity, among others. It is important to carefully monitor patients undergoing CAR-T cell therapy for potential side effects and manage them appropriately to minimize their impact on patient outcomes.[22,23] In this section, we outline and examine the potential side effects that may arise as a result of this novel approach to cancer treatment.

CRS

Immunotherapy aims to activate effector T cells and stimulate them to produce cytokines. Excessive cytokine release can lead to CRS, which varies in severity from mild to severe and can even be fatal.[24] The most commonly observed negative outcome following the administration of CAR-T cells is CRS.[25] CRS can cause a range of symptoms, including high fever, fatigue, nausea, and cardiovascular issues like tachycardia and hypotension.[26,27] In severe cases, CRS can lead to capillary leak, hepatic failure, disseminated intravascular coagulation, and other serious complications. The hallmark indicators of this syndrome typically involve elevated concentrations of certain cytokines in the body, including interferon-gamma (IFN-γ), interleukin-10, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF).[28-31] Notably, patients may still respond to treatment even if they do not experience CRS, particularly in the case of hematologic malignancies.[32] However, most patients with these types of cancer will experience at least mild CRS symptoms, such as fever, after receiving CAR-T cells.[33]

Following a diagnosis of CRS, identifying appropriate medications to alleviate the physiological signs of uncontrolled inflammation while preserving the anti-cancer potency of the modified cells has been challenging.[34] Systemic corticosteroids have been shown to quickly relieve CRS symptoms without jeopardizing the anti-tumor response.[31,35] However, prolonged use of high doses of corticosteroids (for over 14 days) has been linked to the depletion of the CAR-T cells transferred through adaptive means, potentially reducing their long-term anti-leukemia efficacy.[35]

The FDA has approved tocilizumab as an effective treatment for CRS associated with CAR-T cell therapy. This recombinant humanized monoclonal antibody works by blocking the activity of the IL-6 receptor (IL-6R), which is involved in the inflammatory response leading to CRS. Clinical studies have demonstrated that tocilizumab can result in near-immediate reversal of CRS symptoms in patients undergoing CAR-T cell therapy, and it is now widely used as a first-line treatment for this complication.[31,36]

TLS

This syndrome is a potential complication of CAR-T cell therapy that can arise from the breakdown of dying cancer cells.[37] When cancer cells rapidly break down in the body; uric

acid, potassium, and phosphorus levels rise quicker than the kidneys can eliminate them.[38] Excess phosphorus in the body can lead to calcium absorption and lower calcium levels in the blood.[39] Changes in blood levels of these factors can affect the functioning of several organs, including the kidneys, heart, brain, muscles, and gastrointestinal tract.[38] It is important to carefully observe and evaluate patients before and after chemotherapy and CAR-T cell injections.[40] Hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia should be monitored in the blood.[41] Patients should be kept hydrated, and if symptoms indicating the onset of this syndrome develop, they should be treated with allopurinol or rasburicase.[42]

B-cell aplasia

On-target off-tumor toxicity (OTOTT) is an adverse effect caused by the presence of tumor-specific antigens outside of tumor cells.[43] This occurs because CAR-T cells, which are designed to recognize and attack tumor cells, can also target normal cells during the treatment process.[44] CD19, a biomarker for the B-cell lineage, is commonly found on the surface of tumor cells in patients with hematologic malignancies.[45] The use of Anti-CD19 CAR-T cell therapy has demonstrated remarkable success in treating these types of cancers. However, it may lead to the development of B-cell aplasia since the normal B-cells, which also express CD19, can be destroyed during the treatment process.[46] Consequently, treatment can lead to the temporary or permanent loss of B lymphocytes, which the patient may need to receive immunoglobulin periodically.[47]

GVHD

It is a severe and life-threatening complication that requires prompt diagnosis and management.[48] Many patients have received CAR-T cell therapy following allo- hematopoietic stem cell transplantation (HSCT), in which the infused CAR-T cells were from a donor.[49] Despite concerns regarding alloreactivity, studies have shown that CAR-T cells collected after allo-HSCT do not cause GVHD.[29-31] When managing this complication, it is important to consider that immunosuppression can also affect the effectiveness and efficiency of CAR-T cells.[50]

Uncontrolled proliferation

CAR-T cells can proliferate without being regulated by normal control mechanisms.[51] Preclinical studies have shown that CAR-T cells proliferate only in response to physiological signals when exposed to targeted antigens.[52] Nevertheless, T cells can proliferate in response to signals received from malignant tumors or normal B cells, which can be beneficial or harmful depending on the intensity of that proliferation.[53] While CAR-T cell proliferation has been shown to help reduce tumor burden, uncontrolled growth can lead to the excessive accumulation of these cells within the body. In such cases, corticosteroids may be administered to manage and regulate the growth of CAR-T cells. This treatment approach helps prevent potential complications that may arise from uncontrolled growth, ensuring that the patient remains safe throughout the treatment process.[54] Overall, the uncontrolled proliferation of these cells is a potential complication of CAR-T cell therapy. However, preclinical studies have provided insights into the mechanisms behind this proliferation, and strategies are being developed to control it. With continued research, CAR-T cell therapy has the potential to become a powerful tool in the fight against cancer.

Genotoxicity

People who receive genetically engineered products are at risk for genetic modification. This risk is primarily due to viral vectors inserted into the host genome.[55] For example, following the receipt of genetically engineered products and the insertion of a transgene into the genome, a normally functioning gene may be disrupted or a proto-oncogene may be activated, or the regulatory signals may change abnormally.[56] Insertion of retroviral vectors in the vicinity of the LMO-2 oncogene has been associated with most instances of transformation.[57] Moreover, inserting a transgene into T-cells that have already differentiated may heighten the risk of inducing malignant transformation.[58] Nonetheless, so far, there haven't been any cases where modified T-cell infusion has caused transformation. It's worth mentioning that the LMO-2 oncogene is ordinarily inactive in T cells, making it improbable for retroviral integration to occur at this location. The use of genetically modified T cells has been shown to be safe for over ten years, without any indication of vector-induced immortalization, clonal expansion, or a preference for integration sites situated close to genes known to regulate growth or cause transformation.[59] Although the likelihood of oncogenesis resulting from gene alterations in T cells appears to be low, researchers should remain vigilant and consider this possibility.[60]

Neurotoxicity

CAR-T cell treatments can cause neurotoxicity, a severe side effect. Many patients treated with CAR-T cells develop acute neurological signs or symptoms, such as headache, confusion, delirium, language disturbances, seizures, and, in rare cases, severe cerebral edema, which are some of the clinical signs.[61] Regarding the activation and growth of CAR-T cells within a living organism, there is a connection between adverse effects on the nervous system and the release of cytokines, known as CRS.[62] Although the specific mechanisms responsible for this neurological harm are not yet understood, evidence obtained from both animals and humans indicates that the breakdown of the blood-brain barrier is implicated. An increase in cytokine levels present in the blood and cerebrospinal fluid, coupled with elevated activity in endothelial cells, has been linked to this deterioration. Patients experiencing neurotoxicity typically receive treatment comprising corticosteroids, IL-6-targeted therapies, and supportive care. However, there isn't enough high-quality evidence to confirm their effectiveness.[63]

Strategies for Dealing with Toxicity

CAR-T cell therapy has made significant strides in treating cancer in humans, but it has also been linked to certain

harmful effects and distinct toxicities.[64] Traditional anti-cancer therapies do not address the mentioned issues. For this reason, researchers have proposed innovative new approaches to reduce the incidence of toxicities and the severity of their effects. CAR-T cell therapy has been implemented to overcome adverse reactions and reduce the cytotoxicity of these cells to achieve clinical efficacy[65] [Figure 1].

Suicide strategies

To avoid unexpected side effects or eradicate transduced T cells in emergencies such as GVHD incidence or on-target off-tumor toxicity, the use of inducible safety switch genes could be an effective technique.[66] The selective and permanent elimination of CAR-T cells has been the subject of considerable investigation in recent years. Additionally, applying such strategies provides an effective solution to these challenges.[67]

HSV-TK

There has been some research exploring the use of Herpes simplex virus thymidine kinase (HSV-TK) in conjunction with CAR-T cell therapy. In this approach, the HSV-TK gene is inserted into the CAR-T cells themselves, allowing them to be targeted and eliminated if necessary, using ganciclovir (GCV).[68] This strategy can help address some of the safety concerns associated with CAR-T cell therapy, such as CRS or unexpected proliferation. The effectiveness and safety of using combination therapy to treat cancer are still being tested through experiments, so further research is necessary to determine its reliability.[69] The function of the suicide gene is to transform an inactive prodrug into an active and harmful one, which enables GCV to eradicate targeted cells.[70] HSV-TK phosphorylates the GCV and forms GCV-triphosphate, which binds to DNA as a triphosphate substrate. DNA replication is halted through GCV-induced chain termination. The HSV-TK/GCV compound can also trigger signaling cascades by forming Fas-associated death domain protein (FADD), leading to cellular death.[71]

EGFRt and CD20

A different type of safety mechanism for suicide involves modifying T cells to express surface antigens like truncated epidermal growth factor receptor (EGFRt) or CD20, which can be specifically targeted. As a result of this modification, the CAR-T cells are susceptible to either antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).[72] This susceptibility means that the CAR-T cells can be efficiently eliminated by administering monoclonal antibodies such as cetuximab (an EGFR-specific mAb) or rituximab (which is a CD20-specific mAb).[73]

iCasp9

The safety suicide gene known as inducible caspase 9 (iCasp9) is created by fusing human caspase 9 with FK506 binding protein (FKBP), a drug-sensitive protein.[74] When exposed to the chemical inducer of dimerization (AP1903), the fusion protein dimerizes, causing CAR-T cells to undergo apoptosis through the activation of downstream effector caspases.[75,76] The most recent variant of CAR-T cells has been engineered to focus on the CD20 antigen, and it incorporates both a truncated CD19 (ΔCD19) and an iCasp9 suicide gene. These altered T cells can eliminate tumor cells that exhibit CD20, and they could also be employed as a precautionary measure for patients diagnosed with B-cell-related cancers.[75] Administering iCasp9/AP1903 has been proven to effectively eliminate anti-CD20 CAR-T cells in a dose-dependent manner, making it a useful clinical approach.[77] Incorporating the IL-15 gene and an iCasp9-based suicide gene in T lymphocytes that display CAR targeting the CD19 antigen is a novel approach that can effectively enhance the anti-lymphoma/leukemia impact of CAR-T cells in individuals with B-cell-related cancers. This approach has been shown to be safe.[78]

Endogenous switch

iCARs

The inhibitory chimeric antigen receptors (iCARs) were created based on the concept of natural immune-inhibitory receptors found in T-cell regulation.[79] The iCARs have surface antigen recognition components and internal inhibitory signaling domains that come from different receptors such as PD1, CTLA-4, LAG-3, BTLA, and 2B4. These receptors help limit T

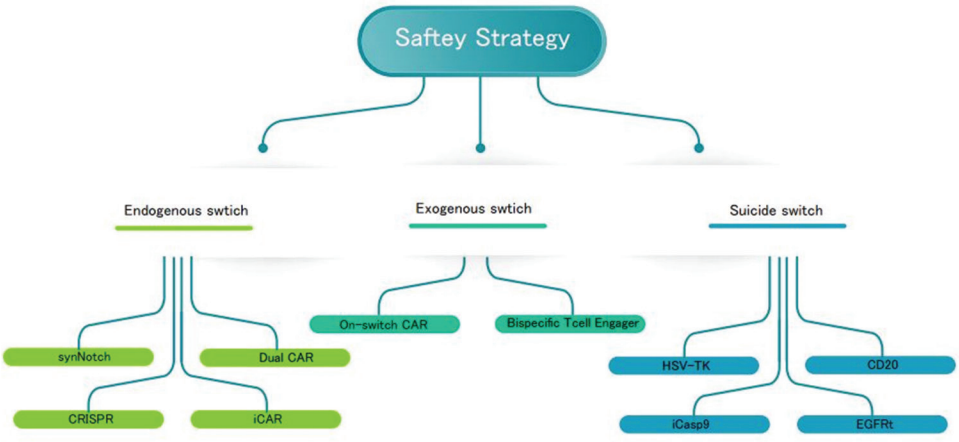

Figure 1: This figure provides an insightful overview of various strategies that can be employed to effectively manage and deal with toxicity associated with CAR T cell therapy

cell responses even when an activating receptor is engaged.[79,80] Studies have shown that human T cells expressing iCARs can reduce cytokine secretion, cytotoxicity, and proliferation driven by endogenous TCRs or the CD19-specific receptor 19-28ζ.[81] In addition, an *in vivo* model was developed to demonstrate that iCAR-expressing T cells spare human cells expressing the iCAR antigen while still eliminating on-target, CD19-positive tumor cells but not off-target CD19 and iCAR antigen dual-positive cells.

Combinatorial targeting tumor-associated antigens

There is a significant desire to create "dual-targeted" T cells that have been altered to express two CARs: one with CD3ζ-linked scFv and another with a chimeric co-stimulatory receptor (CCR) containing CD28 and 4-1BB domains.[75] The co-transduced CAR-T cells could recognize two cancer-specific markers with separate signals and exhibit significant cytotoxicity, with minimal side effects on normal tissues.[82] The use of dual-CARs has advantages in combining different costimulatory domains. In leukemia experiments, using two CARs that target CD19 and CD22 with dual targeting showed better results than administering CD19 or CD22 monospecific CAR-T cells sequentially. A combination of a 28ζ and a BBζ CAR was more effective than having two 28ζ or two BBζ CARs. The best treatment outcomes occurred when the 28ζ CAR targeted CD19 and the BBζ CAR targeted CD22. Shalabi *et al*.[83] also demonstrated the superiority of combining 19.28ζ and 22.BBζ. Ruella *et al*.[13] used a bicistronicity vector encoding CD19 and CD123 BBζ CARs to show that the dual-CAR was superior to pooled CD19 and CD123 CAR-T cells.

In addition, Tan CAR-T cells are designed with two tandem linked scFvs that have specificity for different tumor-associated antigens simultaneously and coupled with the expressing serial CD28 and CD3ζ endo-domains.[84] Based on the strategy, combined target-antigen recognition ensures that CARs are activated only on tumor cells, thereby enhancing anti-tumor accuracy and reducing antigen escape.[85] Recent studies have found that targeting multiple antigens using modified CAR-T cells can be more effective in controlling heterogeneous tumors *in vitro* and *in vivo*.[86] One study found that a combination of CD19 and CD20 using the Tan-19.20 CAR was effective in controlling different types of tumors.[87] In another research, combining BCMA and GPRC5D CARs to target heterogeneous tumors showed that pooled BBζ and dual-BBζ CAR-T cells were more effective than Tan-BCMA.GPRC5D.BBζ counterparts. In preclinical models of glioblastoma, HER2.28ζ and IL13Rα2.28ζ Dual-CAR-T cells were superior to pooled monospecific CAR-T cells.[88] However, an IL13-mutein/HER2.scFv Tan-CAR allowed for more efficient synapse formation and better tumor control.[89] Combining CD19 and CD79a with Tan- or Dual-BBζ CARs was more effective than pooled monospecific CARs for preventing antigen escape, although there were some compromises in antigen binding and signaling.[90] Conversely, the Dual-CAR demonstrated reduced signaling, possibly due to competition for downstream signaling molecules.[89] Dual-CARs that use a common hinge/transmembrane component can heterodimerize and function as parallel CARs.

Synthetic notch receptors

Hacking endogenous T-cell response programs with synthetic Notch (synNotch) receptors provides new and more innovative cellular therapeutics to modulate the activity of the immune system toward cancer.[91] SynNotch circuits, as a programmable platform can be used to flexibly sculpt and customize cellular programs in naive T cells to extend the novel, non-native responses, overcoming tumor immunosuppression.[92] Synthetic Notch-based systems have been developed based on the wild-type Notch protein, consisting of an extracellular antigen recognition domain (e.g., single-chain antibodies or nanobodies), a transmembrane core regulatory domain (TMD), and a synthetic intracellular effector region (e.g., Gal4-VP64).[93] A recent structural study demonstrated that adding a hydrophobic natural motif (QHGQLWF) behind the C-terminus of the Notch core could significantly suppress ligand-independent activation (LIA) of synNotch receptors and refactor T cell functions.[94] Upon SynNotch T cells recognize the specific tumor antigen, conformational changes of the Notch core led to the proteolytic cleavage by the metalloproteinase, releasing the transcriptional regulator from the cytoplasmic tail and then triggering the expression of relevant target genes.[95] The redirected T cells can identify tumor antigens and effectively modify the surrounding microenvironments in a strong and well-regulated manner. The SynNotch receptors have the potential to guide the differentiation of T cells toward Th1 fate choice, leading to the production of IFNγ, which plays a crucial role in eradicating tumors.[91] Customized T cells can locally deliver diverse therapeutic payloads such as cytokines (IL-12 and MIP-1a), antibodies (α-PD-1, CTLA-4, BiTEs), and cytotoxic agents (TRAIL) therefore can minimize toxicity and increase therapeutic efficacy.[96]

CRISPR-associated CAR-T cells

The CRISPR/Cas9 system, which is the ultimate gene editor, has demonstrated remarkable efficacy and enormous potential in adoptive T-cell therapy.[97] This technique has many potential advantages, including high efficiency, flexibility, and multiplex genome editing, and opens a window to developing allogeneic universal CAR-T cells with more optimized structure and reduced toxicities.[98] Table 1 describes the clinical studies on this novel strategy.

Recent breakthroughs in allogeneic gene-edited CAR-T cells can circumvent the constraints and significant barriers such as GVHD and alloreactivity (host versus graft response).[99] CRISPR/Cas9 was used to successfully knock out the endogenous TRAC or TRBC locus (TCRα or TCRβ constant) and HLA-A or Beta-2-Microglobulin (B2M) locus, which was simultaneously replaced by incorporating the CAR cassette to generate double-knockout allogeneic universal anti-CD19 CAR T (DKO UCART19) cells to prevent TCR-induced GVHD, rapid rejection, and the risks of

Table 1: Clinical Trials of CAR-T Cell Therapy with CRISPR-associated safety strategy

Clinical trial Identifier	Status	Target	Gene-knocked Out	Aim of study	Phase	Disease
NCT04037566	Recruiting	CD19	HPK1	Safety	Phase 1	Relapsed/Refractory Leukemia, B-Cell CD19 Positive
NCT04637763	Recruiting	CD19	PD-1	Safety, emerging efficacy, pharmacokinetics, & immunogenicity	Phase 1	Relapsed/Refractory B Cell Non-Hodgkin Lymphoma
NCT05722418	Recruiting	BCMA	TRAC	Safety, effectiveness, determining the best dose	Phase 1	Relapsed/Refractory Multiple Myeloma
NCT05812326	Completed	MUC1	PD-1	Safety, tolerability, & preliminary efficacy	Phase 1 Phase 2	Breast Cancer
NCT04976218	Recruiting	EGFR	TGFβR	Anti-tumor activities & safety	Phase 1	EGFR-positive Solid Tumors
NCT04767308	Not yet recruiting	CD5	CD5	Safety & Efficacy	Early Phase 1	Relapsed/Refractory CD5+Hematopoietic Malignancies
NCT04502446	Recruiting	CD70	CD70	Safety & Efficacy	Phase 1	Relapsed/Refractory T or B Cell Malignancies
NCT05397184	Recruiting	CD3 and CD7	TCR, CD7 & CD52	Safety	Phase 1	Relapsed/Refractory T-cell leukemia
NCT04557436	Recruiting	CD19	CD52 & TRAC loci	Safety & Efficacy	Phase 1	Relapsed/Refractory B Cell Acute Lymphoblastic Leukemia
NCT05643742	Recruiting	CD19	CD19	Safety & Efficacy	Phase 1 Phase 2	Relapsed/Refractory B-cell malignancies.
NCT04438083	Active, not recruiting	CD70	CD83	Safety & Efficacy	Phase 1	Relapsed/Refractory renal cell carcinoma
NCT05795595	Recruiting	CD70	CD70	Safety & Efficacy	Phase 1 Phase 2	Relapsed/Refractory solid tumors

insertional oncogenesis.[15,100] Furthermore, it is advisable to incorporate HLA-E into the engineering of T cells to prevent rejection of CAR-T cells lacking HLA-I by Natural killer (NK) cells.[101]

A recent investigation demonstrated that CRISPR/Cas9-mediated knock-out of GM-CSF significantly reduced the risk of inflammation and CAR-T cell-induced severe CRS as well as enhanced anti-tumor activity.[102] Additionally, disrupting Diacylglycerol Kinase (DGK) in CAR-T cells targeting EGFRvIII could enhance resistance to soluble immunosuppressive factors and promote persistent antigen-specific cytotoxicity.[103] Various studies have demonstrated that the selective elimination of the CD7 gene, prominently expressed in T cells, through CRISPR/Cas9 editing significantly decreased the ability of CAR-T cells to kill other T cells in cases of T-cell acute lymphoblastic leukemia (T-ALL). This led to outstanding and long-lasting clinical responses, while also enhancing anti-cancer immunity and expanding the therapeutic range.[104]

Exogenous switch

Reversible ON-switch CARs

Intracellular micromolecule linkage is a remote-control strategy that addresses a general problem of excessive activity over-engineered therapeutic T cells. Researchers designed robust "ON-switch" CARs utilizing split synthetic receptors.[105] In this control system, the dual-input gated CAR construct resembles a logic gate that distributes key signaling modules from the conventional CAR into two separate components, extracellular single-chain antibody domain (scFv), and intracellular signaling domains. These split chimeric receptors only assemble in the presence of a heterodimerizing small molecule as an exogenous, co-activation signal.[106] The activation of CAR-T cells with molecule-controlled configuration could be controlled precisely by titratable pharmacologic regulation and then mitigating toxicity for durable CAR-T cell therapy.[107]

Juillerat and colleagues suggested the concept of "transient CAR-T cells," which can be activated by the addition of a small molecule drug called AP21967 at specific locations and times. These modified CAR T-cells have multi-chain CARs that allow them to switch on or off the interaction between scFv/antigens and exhibit anti-tumor cytotoxicity.[108] The ability to regulate T cells is critical in enhancing the safety of this technology.[108]

Bispecific T cell-redirecting antibodies

BiTEs are created with the purpose of targeting a specific tumor-associated antigen (TAA) and CD3 co-receptor complexes using two separate scFvs that are linked together by flexible linker peptides.[109] These recombinant antibody-based bifunctional switches have high affinity and flexibility, along with many functional and pharmacological characteristics, which are responsible for CAR-T cell-engaging therapies' long-term and improved efficacy.[110] Peptide-specific switchable CAR-T cells (sCAR-T) have been developed to recognize antigens through binding to the Ab-peptide neo-epitope (PNE) conjugate and the formation of immunological synapses, resulting in MHC-independent cancer cell elimination.[111]

Ab-fluorescein isothiocyanate (FITC) is a platform of bispecific T cell-redirecting antibodies that selectively regulate

and recruit anti-FITC-CAR-T cells against tumor cells.[112] Various antibodies, in particular, trastuzumab, cetuximab, and rituximab could conjugate with FITC as supportive effectors and lead to targeted CAR-T cell-mediated cancer cell killing, along with dose-limiting cytokine release.[113] Blinatumomab, the first-in-class BiTE targeting CD19, has received full approval for the treatment of relapsed or refractory B-cell precursor acute lymphoblastic leukemia (R/R B-ALL) and appears to have curative potential in adult and pediatric patients with minimal residual disease (MRD) who still have detectable cancer traces after chemotherapy.[114] In addition to B-cell malignancies, BiTE therapy might be effective for a wide range of malignancies, including multiple solid cancers, and pave the way for the development of a more effective immunotherapeutic armamentarium.[115]

Conclusion

Genetically engineered CAR-T cell therapy has emerged as a leading option among adoptive cell treatments, particularly for hematopoietic malignancies.[43] The FDA's approval of Tecartus (brexucabtagene autoleucel), a third-generation CAR-T therapy for mantle cell lymphoma (MCL), underscores its potential. However, the therapy is costly, complex to manufacture, and poses safety challenges, making it crucial to manage potential toxicities and identify biomarkers that predict side effects like CRS.[117] Continued analysis of overall survival data is necessary to accurately weigh the long-term benefits and risks associated with CAR-T cell therapy.

To enhance the efficacy of CAR-T therapy, researchers are focusing on identifying robust biomarkers in similar hematological malignancies.[118] Preclinical studies have shown promise with the B cell-activating factor receptor (BAFF-R), which exhibits cytotoxicity against various lymphoma and leukemia cell lines, including CD19-negative variants.[43] As the field progresses, there is a shift toward allogeneic CAR-T infusion due to the high costs and time commitment of traditional methods, with ongoing clinical trials exploring this avenue. Despite the significant advancements in CAR-T cell therapy, challenges remain in managing cytotoxicity and addressing scientific knowledge gaps that hinder widespread adoption.[119] The future will depend on improved medical management of adverse events and innovative gene therapy strategies to determine whether CAR-T therapy can become a first-line treatment for a broader range of hematologic malignancies and other neoplasms in the coming years.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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